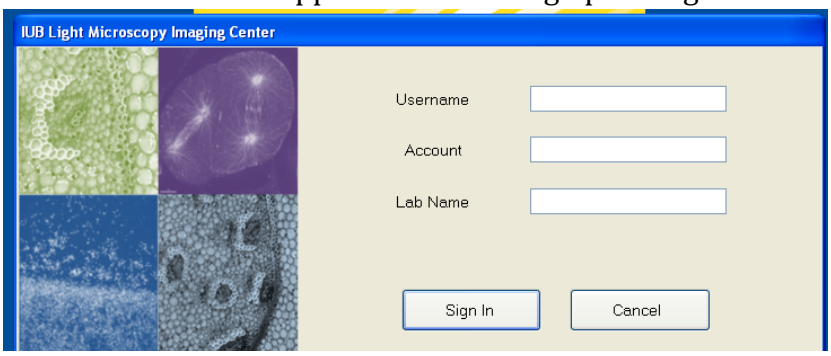
10/2010

LMIC Leica SP5 BASIC use guide (Version 2)

Start-up

- Uncover microscope, tilt illumination arm back, move objectives all the way down (objectives will be down unless you moved them up).
- Turn on the Hg-lamp if you will need it.
- Turn on the laser power switch (switch on the right) and turn on the laser key.
- Turn on Scan box (middle switch).
- Turn on computer/microscope.
- Log-on, form should come up automatically. If computer is already on click on the icon for "shortcut to iCenter application" to bring up the sign-in sheet.



  - add your IU username
  - the account number to be charged
  - and the last name of the lab PI.

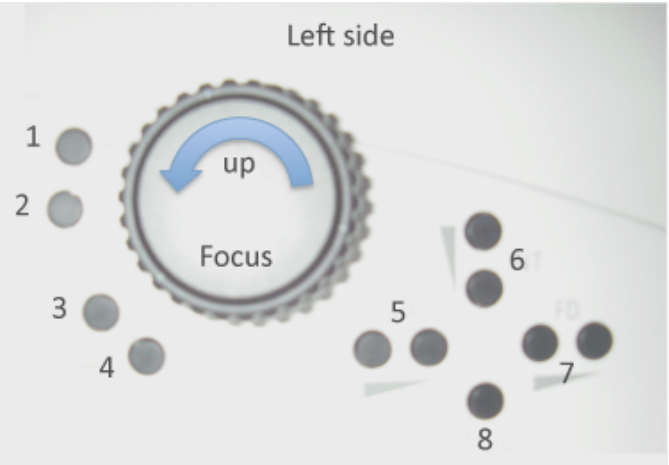
- Start software (LAS AF icon on desktop).
  - Be sure configuration is on "machine".
- After stage initialization, move illumination arm back to upright position.
- You are now ready to look at something.

Microscope

- It only works well when it is clean! Please do your part to keep it clean.
- Insert slide in slot in z-galvo stage insert. (Sample points down toward objective). If you will need oil, put a **small** drop on the coverslip before you load your slide.
    - Avoid getting oil on the non-oil objectives – if you do – ask for help, we will clean the objective.
- Find your sample – I start with the 10X objective (it has a long working distance and won't touch the oil when it is in the right focal plane).

Scope Operation – lots of little buttons. Software controls most things, many are automatic.

Left Side:



1. Change TL – changes transmitted light from brightfield to DIC.
2. Changes to Fluorescence light path – Can also do this by just selecting a filter cube on front panel.
3. Changes filter cubes
4. Change CS – changes to confocal mode
5. Changes aperture diaphragm (automatically adjusted)
6. Light intensity adjustment
7. Field diaphragm – how much of field of view you see.
8. Brightfield light switch

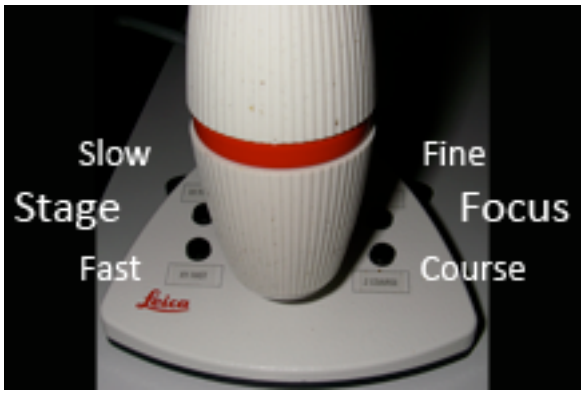
Center:

1. All light to eyepiece.
2. Camera port selection. Will happen automatically.
3. Magnification selection – we don't have this feature – leave button alone.
4. If you press #3, press this button to get back.
5. Fluorescence shutter (makes no noise).
6. Green Fluorescence filter cube
7. Red Fluorescence filter cube
8. Analyzer cube
9. Empty slot for imaging
10. DAPI Fluorescence filter cube
11. Empty slot

Right Side:

1. Press and hold to move objective up to set position.
2. Set button. To set top position, press and hold set button while pressing top button once to clear, again to set.
3. Press and hold to move objective down. **Please don't set bottom position.**
4. Change objective (to left)
5. Change objective (to right)
6. Switch from dry to immersion objective.

Stage/Focus controller (salt & pepper shaker):



- Top – stage movement in X
- Bottom – stage movement in Y
- Back – Focus
- Buttons on left – toggle between fast and precise stage movement.
- Buttons on right – toggle between fine and course focus.

LAS AF Software

- Configuration
    - Turn on lasers you need.
    - Set laser power for Argon laser (20% is a good place to start).
    - Look at objective list – listed for each is the max. resolution. Keep this number in mind when zooming, etc. (It is OK to oversample about 40%).
- Acquisition

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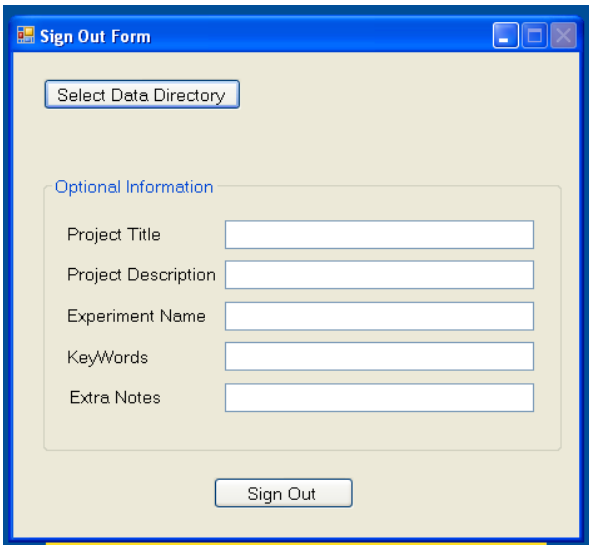
    - Establish light path.
        - The easiest way is to this is to select preloaded Leica settings (red arrow above) that are similar to your needs. This will do many things. It turns on laser controller, laser output. PMTs, and sets windows.
        - Then clink on fluorophore selection bar for each PMT and pick dye you actually used.
        - Adjust collection windows as necessary. **Make sure you are not collecting light over laser output! Window should be at least 10nm away from laser wavelength.**

- Select objective (red circle above). Pay attention to what immersion media each objective requires.
- Pick Acquisition Mode (xyz = "normal"). The others do some nice things. Ask if you need help.
- Format – pick image size. Keep in mind max. resolution of your objective – and remember, smaller is faster.
- Speed – default is 400 Hz and is a good place to start. 600Hz is as fast as it can scan without zooming in. Slower speeds equals better quality and more bleaching.
- See what you have – hit live button – you should get an image. Take time to optimize image – maybe on a spot you don't really care about.
    - Focus – Use the z-control knob on control panel – this is the only focus the computer understands.
    - In general, high gain and low laser power is good. If too grainy, turn laser up. Start with gain around 1000.
    - Check for bleed-through (turn each laser down).
    - Turn on quick-lookup table (rainbow icon on right screen). Blue pixels are maxed out (white), green pixels are black. For best images try a few blue pixels and a few more green (= most dynamic range).
    - Click Zoom, draw box on image where you want to zoom (remember max. resolution).
    - Pinhole is automatically set at optimum – can open it if necessary (more light, less confocal effect).
    - If desired, set Z-series, find top and bottom.
    - Hit **Capture** for single image, or **Start** for series.
- Click Experiment tab. Hit Save All (Until you hit save all – your images will be lost if the computer crashes)

Shut Down

- First turn off key switch for lasers. **Leave laser power switch on!** This turns the lasers off but leaves the cooling fans on to avoid damaging lasers.
- Clean objectives!
- Transfer data to server and sign-out.



- Save all data to transfer to one folder (you can only transfer one folder to server per log-in)
- click "select data directory" button and navigate to your folder.
- Fill in at least "Project Title" so you can find your download on the server.
- Hit "Sign-Out"

Please delete images when you are sure they are safely transferred to the server. You should never have images on the scope computer for more than 1-2 sessions of imaging

- Shut down computer (if you need to work on the computer, you may leave it on).

- Turn off scan head.
- Turn off Hg lamp.
- Cover scope (don't cover Hg lamp).
- Clean up tables, etc.
- When computer screens are dark and the laser fan cable/vent stop shaking (laser cooling fan has stopped) it is safe to turn off power switches to lasers and computer.